level were keeping within the normal range progressed to hepatic failure. The other was that GlaxoSmithKline, U.K., had provided adefovir dipivoxil to Japanese patients with reactivated hepatitis under the condition that one or both of these two variables were out of the normal range of each institution before approval by the Ministry of Welfare and Labor of the Japanese government. This standard for supply of adefovir dipivoxil was established by the cooperation of GlaxoSmithKline and the Japan Society of Hepatology.

Variables Analyzed in this Study

Univariate and multivariate logistic regression analyses were carried out to identify the independent factors associated with the development of hepatic failure caused by lamivudine-resistant HBV. These analyses were performed on the variables at the initiation of the treatment with lamivudine in regard to patients' age, gender, leukocyte and platelet counts in peripheral blood, prothrombin activity, serum levels of albumin, serum levels of total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), rate of serum AST to serum ALT (AST/ALT ratio), serum levels of HBV-DNA and status of HBeAg/Anti-HBe. In the same way, the values at the point of detection of lamivudine-resistant HBV, the duration of the treatment with lamivudine before viral breakthrough, leukocyte and platelet counts in peripheral blood, prothrombin activity, serum levels of albumin, serum levels of total bilirubin, AST, ALT, AST/ALT ratio, serum levels of HBV-DNA and status of HBeAg/Anti-HBe were also analyzed. The maximum levels of ALT after the reactivation of hepatitis and the duration of the follow-up periods from viral breakthrough to the endpoint were also examined. Values of prothrombin activity and the serum levels of total bilirubin at the reactivation of hepatitis were used for the definition of the two groups. But we did not exclude these two variables from logistic regression analyses. The reason was that these two variables used in the analyses were obtained at the initiation of lamivudine therapy and at the detection of lamivudine-resistant HBV before the reactivation.

Statistical Analysis

Univariate logistic regression analysis and multivariate logistic regression analysis were carried out by using the SPSS v.6.1 software for the Macintosh edition (SPSS Inc. Chicago, IL). The Mann-Whitney's U test was used to make comparisons between these two groups for certain variables. A P value of less than 0.05 was considered statistically significant.

Results

The characteristics of the 47 patients at the initiation of lamivudine therapy and those at the point of detection of lamivudine-resistant HBV are shown in Tables 1 and 2, respectively. At the initiation of lamivudine therapy, the mean age of these 47 patients was 46.4 ± 8.0 years. Among them, 33 were male and 14 were female. All had HBV-DNA with an average of 6.98 ± 1.08 LC/ml, and the average of serum ALT was 120 ± 95.7 U/l. Thirty-nine (83.0%) were HBeAg-positive and eight (17%) were HBeAg-negative. The YMDD variant HBV, YIDD or YVDD, were detected in all patients with the SMITEST HBV-YMDD motif ELMA. Twenty-one (44.7%) had an elevation in their serum level of total bilirubin to more than 1.5 mg/dl and/or a decline in the level of prothrombin activity to less than 60% after the reactivation of hepatitis caused by lamivudine-resistant HBV.

Among the variables obtained from the initiation of the treatment with lamivudine, there were significant differences in the patients' age ($P = 0.0473$), platelet count ($P < 0.0001$), prothrombin activity ($P < 0.0001$), AST/ALT ratio ($P = 0.0109$), albumin ($P < 0.0001$) and total bilirubin ($P < 0.0001$) between the two groups (Table 1). As shown in Table 3, there were univariate associations of age at the initiation of lamivudine therapy ($P = 0.0402$), platelet count ($P = 0.0006$), prothrombin activity ($P = 0.0010$), AST/ALT ratio ($P = 0.0076$), serum levels of albumin ($P = 0.0021$), and serum levels of total bilirubin ($P = 0.0039$).

In the analysis of the variables obtained from the data at the point of detection of lamivudine-resistant HBV, platelet count ($P = 0.0002$), prothrombin activity ($P = 0.0104$), serum AST ($P = 0.0084$), ALT ($P = 0.0477$), albumin ($P = 0.0119$), and total bilirubin ($P = 0.0018$) were significantly different between the two groups (Table 2). Univariate logistic regression analysis showed that the variables of platelet count ($P = 0.0013$), prothrombin activity ($P = 0.0141$), albumin ($P = 0.0154$) and bilirubin ($P = 0.0056$) were related to the deterioration of the liver function after the emergence of lamivudine-resistant HBV.

In a multivariate logistic regression analysis using the variables obtained during lamivudine therapy, only prothrombin activity at the initiation of lamivudine therapy was significantly associated with the deterioration of the liver function after the reactivated hepatitis caused by lamivudine-resistant HBV ($P = 0.0025$, 95%CI 0.8269–0.9601) (Table 4). On the other hand, none of the parameters at the point of detection of lamivudine-resistant HBV were associated significantly with the deterioration caused by the drug-resistant HBV. The maximum levels of ALT after the reactivation of hepatitis, the duration of administration of lamivudine before the emergence of

Table 1 Characteristics of patients at the initiation of lamivudine therapy

	All ($n = 47$)	After the reactivation of hepatitis due to LMV-R HBV		P value[a]
		PT act < 60% and/or T-Bil > 1.5 mg/dl ($n = 26$)	PT act ≧ 60% and T-Bil ≦ 1.5 mg/dl ($n = 21$)	
Age (years old)	46.4 ± 8.0	44.2 ± 7.2	49.2 ± 8.2	0.0473
Male sex (%)	33 (70.2%)	17 (65.4%)	16 (76.2%)	0.4256
Peripheral leukocyte (/μl)	5013 ± 1783	5242 ± 1472	4730 ± 2110	0.1774
Peripheral platelet (104/μl)	13.6 ± 6.0	16.6 ± 5.1	9.7 ± 4.6	<0.0001
Prothrombin activity (%)	76.4 ± 22.3	91.7 ± 15.7	60.3 ± 16.1	<0.0001
AST (U/l)	88.6 ± 51.5	86.2 ± 57.6	91.6 ± 44.0	0.363
ALT (U/l)	120 ± 95.7	131.3 ± 103.3	105.9 ± 85.7	0.2798
AST/ALT ratio	0.89 ± 0.40	0.73 ± 0.22	1.09 ± 0.49	0.0109
Serum albumin (g/dl)	3.62 ± 0.68	3.97 ± 0.54	3.20 ± 0.60	<0.0001
Serum total bilirubin (mg/dl)	1.35 ± 1.36	0.73 ± 0.25	2.12 ± 1.74	<0.0001
HBeAg positive	39 (83.0%)	21 (80.8%)	18 (85.7%)	0.6573
HBV-DNA (LC/ml)	6.93 ± 1.08	6.85 ± 1.19	7.02 ± 1.74	0.9339

[a] Mann-Whitney's U test

LC/ml Log copies/ml, *PT act* prothrombin activity, *T-Bil* total bilirubin, *LMV-R* lamivudine-resistant

Table 2 Characteristics of patients during lamivudine therapy and at the point of detection of lamivudine-resistant HBV

	All ($n = 47$)	After the reactivation of hepatitis due to LMV-R HBV		P value[a]
		PT act < 60% and/or T-Bil > 1.5 mg/dl ($n = 26$)	PT act ≧ 60% and T-Bil ≦ 1.5 mg/dl ($n = 21$)	
Variables during lamivudine therapy				
Interval from the initiation of LMV therapy to the detection of LMV-R HBV (day)	523 ± 248	474 ± 250	584 ± 237	0.0642
Interval from the detection of LMV-R HBV to the last observation (day)	456 ± 299	483 ± 294	423 ± 310	0.4868
Maximum levels of ALT after Viral breakthrough (U/l)	274.9 ± 246.8	270.3 ± 215.6	280.6 ± 286.2	0.7563
Variables at the point of detection of lamivudine-resistant HBV				
Peripheral leukocyte (/μl)	4761 ± 1585	5012 ± 1792	4450 ± 1258	0.1915
Peripheral platelet (10^4/μl)	14.4 ± 5.6	17.1 ± 4.8	11.1 ± 4.7	0.0002
Prothrombin activity (%)	87.0 ± 16.6	95.1 ± 10.9	80.2 ± 17.8	0.0104
AST (U/l)	56.3 ± 70.8	58.3 ± 93.1	53.9 ± 26.2	0.0084
ALT (U/l)	65.7 ± 115.4	68.3 ± 150.7	62.5 ± 46.7	0.0477
AST/ALT ratio	1.07 ± 0.45	1.07 ± 0.46	1.06 ± 0.46	0.6454
Serum albumin (g/dl)	4.13 ± 0.76	4.40 ± 0.34	3.79 ± 0.98	0.0119
Serum total bilirubin (mg/dl)	0.95 ± 0.50	0.74 ± 0.29	1.20 ± 0.59	0.0018
HBeAg positive	38 (80.6%)	21 (80.8%)	17 (81.0%)	0.7511
HBV-DNA (LC/ml)	5.63 ± 1.24	5.68 ± 1.21	5.58 ± 1.31	0.8071

[a] Mann-Whitney's U test

LC/ml Log copies/ml, *PT act* prothrombin activity, *T-Bil* total bilirubin, *LMV-R* lamivudine-resistant

lamivudine-resistant HBV, and the duration of the follow-up period after the detection of lamivudine-resistant HBV were not significantly associated with the progression of the disease.

Discussion

In a clinical trial of patients with a compensated liver function, lamivudine suppressed the replication of HBV-

Table 3 Univariate logistic regression analysis

	P	Odds ratio	95% Confidence interval
Variables at the initiation of lamivudine therapy			
Age (years old)	0.0402	1.0957	1.004–1.1958
Male sex (%)	0.4228	1.694	0.4668–6.1472
Peripheral leukocyte (/μl)	0.3262	0.9998	0.9994–1.0002
Peripheral platelet (10^4/μl)	0.0006	0.738	0.6206–0.8776
Prothrombin activity (%)	0.001	0.8789	0.8137–0.9493
AST (U/l)	0.7212	1.0021	0.9908–1.0136
ALT (U/l)	0.3685	0.997	0.9906–1.0035
AST/ALT ratio	0.0076	18.1342	2.1615–152.1386
Serum albumin (g/dl)	0.0021	0.0977	0.0239–0.3986
Serum total bilirubin (mg/dl)	0.0039	23.35	2.7464–98.526
HBeAg positive	0.6548	1.4286	0.2999–6.8236
HBV-DNA (LC/ml)	0.6011	1.1614	0.6674–2.0208
Variables during lamivudine therapy			
Interval from the initiation of LMV therapy to the detection of LMV-R HBV (day)	0.1389	1.0019	0.9993–1.0045
Interval from the detection of LMV-R HBV to the last observation (day)	0.4922	0.9993	0.9973–1.0012
Maximum levels of ALT after Viral breakthrough (U/l)	0.886	1.002	0.9996–1.0043
Variables at the point of detection of lamivudine-resistant HBV			
Peripheral leukocyte (/μl)	0.2312	0.9998	0.9994–1.0002
Peripheral platelet (10^4/μl)	0.0013	0.7665	0.6521–0.901
Prothrombin activity (%)	0.0141	0.9293	0.8764–0.9854
AST (U/l)	0.8277	0.9991	0.9907–1.0076
ALT (U/l)	0.8614	0.9995	0.9944–1.0046
AST/ALT ratio	0.9111	0.9294	0.2571–3.3587
Serum albumin (g/dl)	0.0154	0.12	0.0216–0.6668
Serum total bilirubin (mg/dl)	0.0056	17.5227	2.3127–132.7638
HBeAg positive	0.7487	1.2631	0.3025–5.2742
HBV-DNA (LC/ml)	0.7911	0.9377	0.5829–1.5086

LC/ml Log copies/ml

Table 4 Multivariate logistic regression analysis of variables

Variables	*P*	Odds ratio	95% Confidence interval
Prothrombin activity at the initiation of LMV therapy (%)	0.0025	0.891	0.8269–0.9601

DNA sufficiently in more than 90% of them and improved the serum ALT levels as well as liver histology [11]. Lamivudine is generally well tolerated with few adverse events, and hepatitis flares are uncommon during treatment unless the drug-resistant HBV emerges. These features make treatment with lamivudine more feasible compared with IFN for patients with decompensated liver cirrhosis. The beneficial effects of lamivudine therapy were observed in patients with a compensated liver function as well as in patients with decompensated liver cirrhosis. Lamivudine rapidly suppressed the replication of HBV-DNA and improved the biochemical parameters in some clinical trials for HBV-related decompensated liver cirrhosis [24–29].

However, the incidence of lamivudine-resistant HBV in patients with decompensated liver cirrhosis varied from 7 to 21% [24, 25, 27, 30]. The reactivation of hepatitis due to lamivudine-resistant HBV is often associated with a fatal outcome, especially when the drug-resistant HBV emerged in patients with liver cirrhosis [24, 25, 27, 30]. The factors associated with the emergence of lamivudine-resistant HBV were commented on in many previous reports. In this study, we detected predictive factors associated with the prognosis after the emergence of lamivudine-resistant HBV, and which were the parameters obtained from the data at the introduction of lamivudine. It might be meaningful that the predictive factors were detected among the

baseline parameters, because patients with a history of a decompensated liver function were at greater risk of a fatal outcome. We showed that prothrombin activity at the initiation of lamivudine had a significant association with the deterioration of liver function caused by lamivudine-resistant HBV. The liver is the major site of synthesis of coagulation proteins. Prothrombin is the independent predictive index of hepatic fibrosis [31]. Among the variables obtained at baseline, univariate logistic regression analysis showed that platelet count, AST/ALT ratio, albumin and total bilirubin were significantly associated with hepatic failure caused by lamivudine-resistant HBV in addition to prothrombin activity, although there was no significance by multivariate logistic regression analysis. Previous studies reported that these parameters were also related to the progression of chronic liver disease [32–35]. Except for the patients' age, five of six variables that were extracted as significant variables by univariate logistic regression analysis reflected the reserve capacity of the liver rather than the activity of hepatitis.

In this study, we showed that the maximum levels of ALT after the reactivation of hepatitis due to lamivudine-resistant HBV did not have significant prognostic relevance with regard to the deterioration of the liver function. In a clinical situation, the maximum levels of ALT were not always higher in patients who developed hepatic failure after the selection of lamivudine-resistant HBV. For these reasons, we estimated that these results emphasized that the deterioration of the liver function caused by lamivudine-resistant HBV was more closely related with the reserve capacity of the liver before the initiation of treatment with lamivudine. Moreover, this result suggests that the patients whose disease had already progressed to decompensated liver cirrhosis at the initiation of lamivudine therapy should be observed more carefully than patients with compensated HBV infection, even those whose liver function had improved by the administration of lamivudine.

We reached the conclusion that reactivated hepatitis was not severe in patients whose liver function was compensated at baseline. On the other hand, those with liver disease which had progressed were at risk of hepatic failure. In such patients, in order to prevent the progression to hepatic failure, additional or alternative treatment with another antiviral agent should be recommended, even though the hepatitis has not yet been reactivated.

Several new nucleoside analogues are now under development and adefovir dipivoxil is a new-arrival agent in the clinical field. So far, the approved nucleoside analogues are limited to two kinds of medicine, lamivudine and adefovir dipivoxil. The active metabolite of adefovir dipivoxil, adefovir diphosphate, displays potent antiviral activity against HBV [36, 37], and an in vitro study demonstrated the antiviral activity to be against both wild-type and lamivudine-resistant strains of HBV [38]. Recent clinical trials reported that adefovir dipivoxil showed sufficient antiviral efficacy both in patients with wild-type HBV and lamivudine-resistant HBV [39, 40] with the advantage of a low incidence of drug-resistant HBV-DNA mutations [41]. In patients with compensated liver disease, adefovir dipivoxil alone or in combination with ongoing lamivudine therapy provided sufficient antiviral effects [42], although a small portion of patients treated with adefovir dipivoxil alone had an elevation of serum ALT levels after the cessation of lamivudine therapy [42]. In patients with decompensated liver cirrhosis, a mild flare of ALT triggers hepatic failure. For these reasons, we think that the combination therapy with adefovir and lamivudine should be continued until HBV-DNA level decreases and the activity of reactivated hepatitis is suppressed sufficiently in patients with liver cirrhosis.

Entecavir is approved for the treatment of chronic hepatitis B by FDA based on the three phase III trials [43–45]. New nucleoside analogue that have sufficient antiviral effects on both wild-type HBV and lamivudine-resistant HBV, such as tenofovir, are now under clinical trials for the treatment of chronic HBV infection [46]. It was reported that these medicines had the advantage of a lower incidence of the drug-resistant HBV variants [46, 47].

In conclusion, the prognosis after the emergence of lamivudine-resistant HBV was more closely related to the reserve capacity of the liver at the initiation of lamivudine therapy than the activity of reactivated hepatitis. Therapies for HBV-related chronic liver disease should be tailored according to the stage of the liver disease as well as to viral and host factors. From the foregoing, it would appear that additional treatment with adefovir dipivoxil or another antiviral agent early after viral breakthrough should be recommended when the lamivudine-resistant HBV is detected in patients with the history of decompensated liver disease, even before ALT is elevated. And these patients should be observed more carefully and strictly, even if the liver function improves after the administration of lamivudine.

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HEPATOLOGY

Insulin resistance and lichen planus in patients with HCV-infectious liver diseases

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Abstract

Background and Aim: Hepatitis C virus (HCV) causes liver diseases and extrahepatic manifestations, and also contributes to insulin resistance and type 2 diabetes mellitus (DM). The aims of the present study were to examine the incidence of extrahepatic manifestations including lichen planus in HCV-infected patients and to evaluate the relationship between lichen planus and insulin resistance.

Methods: Of 9396 patients with liver diseases presenting to the study hospital, 87 patients (mean age 60.0 ± 11.5 years) with HCV-related liver diseases were identified and examined for the incidence of extrahepatic manifestations. Insulin resistance and the presence of *Helicobacter pylori* antibodies were also measured.

Results: The prevalence of DM was 21.8% (19/87), hypertension was 28.7% (25/87), thyroid dysfunction was 20.7% (18/87), and extrahepatic malignant tumor was 9.2% (8/87). The prevalence of lichen planus at oral, cutaneous, pharyngeal, and/or vulval locations was 19.5% (17/87). Characteristics of 17 patients with lichen planus (group A) were compared with 70 patients without lichen planus (group B). Prevalence of smoking history, presence of hypertension, extrahepatic malignant tumor, and insulin resistance (HOMA-IR) were significantly higher in group A than in group B. Significant differences were not observed for age, sex, body mass index, diagnosis of liver disease, alcohol consumption, presence of DM, thyroid dysfunction, liver function tests, or presence of *H. pylori* infection between the two groups.

Conclusions: Infection with HCV induces insulin resistance and may cause lichen planus. It is necessary for an HCV-infected patient to be assayed for insulin resistance, and to be checked for different extrahepatic manifestations of this infection, particularly lichen planus.

Introduction

The number of fatalities due to hepatocellular carcinoma (HCC) in Japan continues to increase, and it is estimated that this tendency will continue at least until 2015. Of the HCC cases in Japan, approximately 16% are caused by hepatitis B virus (HBV) infection and approximately 80% by hepatitis C virus (HCV) infection.[1] The average prevalence of HCV carriers in Japan is about 2%, with the absolute number estimated at 2 million.[2] The increase in HCC in Japan depends on the spread of HCV infection.[2]

Infection with HCV induces various extrahepatic manifestations as well as chronic liver diseases.[3,4] HCV infects cells or organs except hepatocytes and multiplies. Representative extrahepatic manifestations of HCV infection include lichen planus, diabetes mellitus (DM), malignant lymphoma, Sjögren's syndrome, cryoglobulinemia, and membranoproliferative glomerulonephritis. It has been reported that combined therapy using interferon and ribavirin is effective for different extrahepatic manifestations that are apt to be overlooked.[5,6]

At present, it has been shown that HCV multiplies in skin and oral mucosa leading to HCV-related lichen planus,[7,8] and that the risk of malignant transformation is higher in lichen planus with HCV infection than in lichen planus without HCV.[9] However, a mechanism for these extrahepatic manifestations has not been elucidated. Recently it was reported that there is a significant correlation between lichen planus and HCV and DM in southern Taiwan, particularly in HCV patients with elevated serum alanine aminotransferase (ALT) levels and atrophic-erosive oral lichen planus (OLP).[10] In our previous report, patients with lichen planus having DM were all found to be HCV-infected.[11]

In addition, it has been reported that DM is a risk factor for HCV-related hepatocarcinogenesis[12] and for decreased survival

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among liver cirrhosis patients.[13] In addition, the incidence of diabetes in patients having HCV-related liver cirrhosis is higher than that in patients with HBV-related liver diseases.[14]

We recently showed molecular mechanisms for HCV core-induced insulin resistance.[15] HCV core up-regulates the suppressor of cytokine signaling (SOCS) 3, and inhibits insulin signaling by down-regulation of insulin receptor substrate (IRS) -1 and IRS-2 in hepatocytes. Moreover, in an epidemiological survey, we demonstrated that a significant increase in the incidence of diabetes occurs in subjects with high titers of HCV core compared to subjects who are negative for anti-HCV antibody[16] and concluded that HCV infection induces insulin resistance, which causes an increase in the incidence of extrahepatic manifestations in HCV-infected individuals.[17]

In the current study, we surveyed the incidence of abnormal glucose tolerance in patients with or without lichen planus in a study population with HCV-related chronic liver disease, and investigated the relationship between lichen planus and insulin resistance.

Methods

Patients

A total of 105 984 consecutive patients had checkups for chronic liver disease for the first time in the Digestive Disease Center at Kurume University Hospital from April 1988 to August 2005. In the Digestive Disease Center, physicians, surgeons, radiologists, and an oral surgeon hold full-time positions. One of us (M.S.) is a hepatologist and examined 9396 of these 105 984 patients. There were 522 patients who were HCV antibody positive and who thereafter continued with regular hospital visits until April 2006.

Exclusion criteria were the following: (i) other causes of chronic liver disease or disease other than chronic HCV infection; (ii) liver disease related to HBV infection; and (iii) patients treated with interferon therapy at the time of study inclusion.

We examined the presence of extrahepatic manifestations of chronic HCV infection in 87 patients. Informed consent was obtained from all patients after the purpose and methods of the study were explained. The 87 patients were 44 men and 43 women with a mean age of 60.0 ± 11.5 years.

The patients were monitored for the presence of extrahepatic manifestations of HCV infection such as lichen planus, DM, hypertension, thyroid dysfunction, and extrahepatic malignant tumor as well as liver disease. Biochemical tests were done and insulin values, blood glucose levels, and *Helicobacter pylori* antibody were measured in patient blood samples. Life histories were taken.

Clinical examinations

Patients received oral mucosa and cutaneous medical examinations by an oral surgeon and a dermatologist. The diagnosis of OLP was made on the basis of clinical and histopathological features. Diagnosis of type 2 DM was based on the American Diabetic Association (ADA) criteria of 1997.[18] Persons in whom diabetes was diagnosed before 30 years of age and who used insulin were categorized as type 1 DM and were excluded from our study.

The following definitions of cardiovascular disease were employed. Obesity was defined as a body mass index (BMI) >25 kg/m^2 or higher. Hypertension was defined as a systolic blood pressure (SBP) of 140 mmHg or higher, or a diastolic blood pressure (DBP) of 90 mmHg or higher according to the criteria of JNC-VI of the International Hypertension Society.[19] Thyroid hormones such as FT3, FT4 and thyroid stimulating hormone were measured for all patients, and thyroid echography examination was performed for some patients. Examination of the upper gastrointestinal tract or lower digestive tract was performed on patients for whom it was deemed clinically necessary.

We also took a history of smoking and alcohol consumption.

Serological assays

Serum samples from the 87 patients were collected and tested for platelets (PLT) and for the following liver function tests: serum ALT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), total bilirubin (TBil), direct bilirubin (DBil), thymol turbidity test (TTT), zinc sulfate turbidity test (ZTT), total cholesterol (TC), total protein (TP), and albumin (Alb). Sera were also examined for the presence or absence of HCV or HBV infection. Anti-HCV was measured by a chemiluminescent enzyme immunoassay kit (Lumipulse II HCV, Fujirebio, Tokyo, Japan). HCV RNA in serum was detected using the Amplicore HCV test (Roche, Tokyo, Japan). Hepatitis B virus surface antigen (HBsAg) was assayed using a chemiluminescent immunoassay kit (Architect, HBsAg QT, Dainabot, Tokyo, Japan). Ultrasonographic examination for all patients was performed in order to investigate the shape of the liver and lesions occupying the liver. Computed tomography and liver biopsy were performed in some patients. Most patients underwent endoscopy for detection of esophagogastric varices. We used other possible predictors of liver cirrhosis progression, including serum albumin, TBil, prothrombin time, and PLT.

Plasma glucose levels were measured by a glucose oxidase method for all subjects and serum insulin levels were measured using a sandwich enzyme immunoassay kit (Eiken Chemical, Tokyo, Japan). Insulin resistance (IR) was calculated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA-IR) method.[20] The formula for the HOMA-IR is: HOMA-IR = fasting glucose (mg/dL) × fasting insulin (μU/mL)/405.

The presence of serum IgG antibodies against *H. pylori* antibody were measured by the SRL (Tokyo) using E Plate *H. pylori* antibody produced by Eiken Chemical.

Statistical analysis

The chi-squared test and the unpaired Student *t*-test were used for statistical analyses. Differences were judged significant for $P < 0.05$ (two-tailed). This study was approved by the Institutional Review Board/Ethics Committee of our Institution.

Results

Among 87 patients with HCV-related liver diseases, the prevalence of lichen planus was 19.5% (17/87), DM was 21.8% (19/87),

Table 1 Clinical characteristics of 87 patients with HCV-related liver diseases according to presence of lichen planus (LP)

Clinical characteristic	All patients	Group A (with LP)	Group B (without LP)	*P*-value (A *vs* B)
No. subjects	87	17	70	–
Age (years)	60.0 ± 11.5	63.7 ± 10.6	59.1 ± 11.6	NS
Sex (M/F)	44/43	11/6	33/37	NS
BMI (kg/m^2)	22.8 ± 2.9	23.9 ± 2.8	22.5 ± 2.9	NS
Smoking history	32 (36.8)	10 (58.8)	22 (31.4)	0.0356
Alcohol consumption percentage	50 (57.5)	10 (58.8)	40 (57.1)	NS
Diagnosis of liver disease				
Past history of HCV infection	1	0	1	NS
Chronic hepatitis C	69	11	58	
HCV-related liver cirrhosis	9	3	6	
HCV-related HCC	8	3	5	
Comorbidities				
Diabetes mellitus	19 (21.8)	4 (23.5)	15 (21.4)	NS
Hypertension	25 (28.7)	10 (58.8)	15 (21.4)	0.0022
Thyroid dysfunction	18 (20.7)	5 (29.4)	13 (18.6)	NS
Extrahepatic malignant tumor	8 (9.2%)	5 (29.4)†	3 (4.3)‡	0.0013

Values shown as *n* (%) or mean ± SD. BMI, body mass index; F, female; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M, male; NS, not significant.

†Tumors were: gastric cancer (two), tongue cancer (one), larynx cancer (one), and renal and colon cancer (one). ‡Tumors were: gastric cancer (one), colon cancer (one), and gallbladder cancer (one).

hypertension was 28.7% (25/87), thyroid dysfunction was 20.7% (18/87), and extrahepatic malignant tumor was 9.2% (8/87).

We compared characteristics of 17 patients who had lichen planus (group A) and 70 patients who did not have lichen planus (group B). The mean age in group A was 63.7 ± 10.6 years; there were 11 men and six women. The mean age in group B was 59.1 ± 11.6 years; there were 33 men and 37 women. Table 1 shows clinical features of groups A and B. The diagnoses of liver diseases in group A were chronic hepatitis C infection (11 patients), HCV-related liver cirrhosis (three patients), and HCV-related HCC (three patients). Those of group B were chronic hepatitis C infection (58 patients), HCV-related liver cirrhosis (six patients), HCV-related HCC (five patients) and past history of HCV infection (one patient) (Table 1).

The prevalence of smoking history ($P = 0.0356$), hypertension ($P = 0.0022$), and extrahepatic malignant tumor ($P = 0.0013$) were significantly higher in group A than in group B (Table 1). Diagnoses of extrahepatic malignant tumors in group A were: tongue cancer (one squamous cell carcinoma), larynx cancer (one squamous cell carcinoma), gastric cancer (one adenocarcinoma, one signet ring cell carcinoma), renal and colon cancer (one renal cell carcinoma). Diagnoses of extrahepatic tumor in group B were: gastric cancer (one adenocarcinoma), colon cancer (one adenocarcinoma), and gallbladder cancer (one adenocarcinoma). Significant differences were not observed for age, sex, BMI, liver disease, alcohol consumption, presence of DM, or thyroid dysfunction between these two groups.

We analyzed for differences between these two groups in liver assays, blood platelets, insulin, blood glucose, HOMA-IR, and presence of *H. pylori* infection. The laboratory data of both groups are shown in Table 2. Prevalence of insulin ($P = 0.0076$) and HOMA-IR ($P = 0.0113$) were significantly higher in group A than in group B (Table 2). Significant differences were not observed for serum AST, ALT, LDH, γGTP, TP, Alb, TBil, DBil, TTT, ZTT, TC, blood platelets, blood glucose, or presence of *H. pylori* infection between these two groups.

Seventeen patients had OLP at a total of 24 sites. The site of occurrence was: buccal mucosa in 13 (76.5%), lower lip in six (35.3%), upper lip in two (11.8%), gingiva in one (5.9%), tongue in one (5.9%), and floor of mouth in one (5.9%) (Table 3). The sites of lichen planus except oral mucosa were lower leg in four (23.5%), antebrachium in one (5.9%), skin extremities in two (11.8%), hypopharynx in one (5.9%), and vulva in one (5.9%). Biopsies of hypopharyngeal lichen planus were performed by an otolaryngologist, and of vulvar lichen planus by a gynecologist. The erosive and reticular variety, respectively, was found to be the prevalent form (Table 3).

Discussion

We performed an epidemiological survey for extrahepatic manifestations and HCC in an HCV hyperendemic area in Japan.[21,22] Anti-HCV positivity among residents of this area in 1990 was 23.6%.[23] We found that the prevalence of extrahepatic manifestations among individuals with HCV infection was higher than among those without HCV,[22] and found an association between HCV core, insulin resistance, and the development of type 2 DM.[16] Recently, we reported that insulin resistance in inhabitants who have an extrahepatic manifestation including OLP with HCV infection shows significantly greater increases than for inhabitants who have neither an extrahepatic manifestation nor HCV infection.[17] By the results of these epidemiological surveys we think that insulin resistance induced by HCV infection causes an increase in the incidence of extrahepatic manifestations in HCV-infected individuals.

In this study, we did long-term follow up for insulin resistance from the standpoint of lichen planus among patients who we identified as having HCV-related chronic liver disease at our hos-

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Table 2 Laboratory data of 87 patients with HCV-related liver diseases according to presence of lichen planus (LP)

Laboratory assay	All patients	Group A (with LP)	Group B (without LP)	*P*-value (A *vs* B)
AST (IU/L)	61.1 ± 38.1	60.9 ± 33.5	61.2 ± 39.3	NS
ALT (IU/L)	68.2 ± 46.7	62.4 ± 39.6	69.6 ± 48.5	NS
LDH (IU/L)	216.8 ± 62.8	205.8 ± 72.1	219.6 ± 60.6	NS
γ-GTP (IU/L)	64.1 ± 68.4	63.5 ± 50.0	64.2 ± 72.5	NS
TP (g/dL)	7.7 ± 0.5	7.7 ± 0.5	7.7 ± 0.5	NS
Alb (g/dL)	4.1 ± 0.5	3.9 ± 0.5	4.2 ± 0.5	NS
PLT (/mm^3)	13.8 ± 5.1	12.5 ± 5.0	14.1 ± 5.09	NS
TBil (mg/dL)	1.1 ± 0.6	1.2 ± 0.9	1.0 ± 0.5	NS
DBil (mg/dL)	0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.2	NS
TTT	16.2 ± 6.7	18.4 ± 4.7	15.8 ± 7.0	NS
ZTT	20.6 ± 6.9	21.8 ± 5.8	20.3 ± 7.2	NS
TC (mg/dL)	172.3 ± 35.8	164.3 ± 41.9	174.1 ± 34.4	NS
Insulin (μU/L)	23.3 ± 42.0	47.3 ± 87.8	17.4 ± 15.4	0.0076
Blood glucose (mg/dL)	97.4 ± 30.1	103 ± 33.2	96.1 ± 29.5	NS
HOMA-IR	7.1 ± 18.8	17.4 ± 40.0	4.6 ± 6.0	0.0113
Helicobacter pylori antibody (*n* (%))	58 (66.7)	10 (58.8)	48 (68.6)	NS

Values shown as mean ± SD. Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DBil, direct bilirubin; γ-GTP, gamma-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment; LDH, lactate dehydrogenase; NS, not significant; PLT, platelets; TBil, total bilirubin; TP, total protein; TTT, thymol turbidity test; TC, total cholesterol; ZTT, zinc sulfate turbidity test.

Table 3 Location of lichen planus in 17 patients with hepatitis C virus-related liver diseases

No	Sex	Age (years)	Liver disease	Lichen planus location			Type
				Cutaneous	Oral	Other	
1	M	71	CH	Antebrachium	–	–	–
2	M	60	CH	Extremities	–	–	
3	F	70	LC	–	Gingiva	–	Erosive
4	M	72	LC	–	Lower lip	–	Reticular
5	F	64	LC	Leg	Buccal mucosa, upper lip, lower lip	–	Erosive
6	M	66	CH	Leg	Buccal mucosa, upper lip, lower lip	–	Erosive
7	M	59	CH	–	Buccal mucosa (reticular)	Pharynx (erosive)	Erosive + reticular
8	M	66	CH	Leg	Buccal mucosa, lower lip	–	Reticular
9	M	57	CH	–	Buccal mucosa	–	Reticular
10	M	50	CH	–	Buccal mucosa, tongue, lower lip	–	Erosive
11	F	77	CH	–	Buccal mucosa	–	Atrophic
12	F	75	CH	–	Buccal mucosa	–	Reticular
13	M	62	HCC	–	Buccal mucosa, lower lip	–	Erosive
14	F	83	HCC	Leg	Buccal mucosa (atrophic)	Vulva (erosive)	Atrophic + erosive
15	M	41	CH	–	Buccal mucosa	–	Reticular
16	M	58	HCC	Extremities	Buccal mucosa, floor of mouth	–	Erosive
17	F	53	CH	–	Buccal mucosa	–	Reticular

CH, chronic hepatitis C; F, female; LC, HCV-related liver cirrhosis; HCC, HCV-related hepatocellular carcinoma; M, male.

pital. Although there was no significant difference in fasting glucose levels and BMI between patients with and without lichen planus, fasting insulin levels and HOMA-IR values, an indicator of insulin resistance, were significantly higher in patients who had lichen planus than in those who did not.

In the present study, insulin levels (17.4 ± 15.4 μU/L) and HOMA-IR values (4.6 ± 6.0) in patients having HCV infection without lichen planus (group B) were higher than the normal range. Normal values for insulin are 3.06–16.9 μU/L, and for HOMA-IR are less than 2. Therefore, the significantly higher insulinemia in patients such as those in group A (among HCV infectious patients) might cause lichen planus.

In Japan, it is known that the prevalence of HCV infection in patients with lichen planus is high;[11] therefore, interferon therapy is often administered to patients with lichen planus and a persistent HCV infection. However, it has been reported that patients cannot

complete interferon therapy because of aggravation of lichen planus.[24,25] The measurement of insulin resistance as well as a search for lichen planus may be useful before performing interferon therapy. A large series of patients with OLP was evaluated for extraoral involvement by Eisen *et al.*[26] They concluded that any patient with OLP should undergo a through history and examination as part of an investigation of potential extraoral manifestations, because a high percentage of patients with OLP develop extraoral manifestations. In our 17 cases of lichen planus, cutaneous lichen planus was diagnosed in seven (41.2%), hypopharynx in one (5.9%), and vulva in one (5.9%). The simultaneous appearance of extraoral and oral lesions was noted among six (35.3%). Because the majority of OLP patients suffer from lichen planus of the genitalia,[27] clinicians should follow OLP patients with sufficient attention to the presence of extraoral manifestations.

Sikuker *et al.* evaluated an association between HCV infection and extrahepatic malignancies. Extrahepatic malignancies were found in 14.6% of anti-HCV positive patients.[28] The incidence of extrahepatic malignant tumor in our subjects was 9.2% (8/87). The insulin-like growth factor family of proteins plays a key role in cellular metabolism, differentiation, proliferation, transformation and apoptosis, during normal development and malignant growth.[29] The hyperinsulinemia that HCV infection causes may induce an extrahepatic malignant tumor as well as HCC.

Many studies have shown that *H. pylori* is involved in the pathogenesis of gastric cancer.[30] The seroprevalence of *H. pylori* is 71% in Japanese aged 50–59 years, and is 81% in those aged 60–69 years.[31] This is almost the same as the seroprevalence of our patients, which was 66.7% (58/87) overall and 82.6% (19/23) in those aged 60–69 years. Seroprevalence of *H. pylori* in our three subjects with gastric cancer was 66.7%. In our study, we did not find an association between *H. pylori* and lichen planus in patients with HCV-infectious liver diseases.

In conclusion, we investigated the association of insulin resistance and lichen planus among patients with HCV-infected chronic liver diseases. The significant factors for development of lichen planus were smoking history, presence of hypertension, extrahepatic malignant tumor, and insulin resistance (HOMA-IR). This supports our previous conclusion that insulin resistance in patients who have an extrahepatic manifestation of HCV infection increases more than insulin resistance of patients who have neither an extrahepatic manifestation nor HCV infection. HCV-infected patients with lichen planus should pay attention to the development of an extrahepatic malignancy. Cooperation with an oral surgeon and a hepatologist is vital for early diagnosis and treatment of any extrahepatic manifestations.

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HBV and HCV infection in Japanese dental care workers

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Abstract. Protective measures against occupational exposure to the hepatitis B virus (HBV) and hepatitis C virus (HCV) must be taken in order to prevent infection in dental care workers. To determine the best way to protect these workers, our study examined viral hepatitis infection in dental care workers in regions with a high prevalence of HCV infections in Japan. In total, 141 dental care workers (including dentists, dental hygienists and dental assistants) were enrolled. After a questionnaire to elicit demographic information was administered by an oral surgeon, hepatitis B surface antigen (HBsAg), antibody to HBs (anti-HBs), antibody to hepatitis B core antigen (anti-HBc) and antibody to HCV (anti-HCV) were measured. When necessary, HBeAg, anti-HBe, levels of HBV DNA, anti-HBc IgM and HCV RNA in serum were measured. Of the dental care workers included, 68 (48.2%) had been immunized with a HBV vaccine. Only 9 wore a new pair of gloves for each new patient being treated, 36 changed to a new pair only after the old gloves were torn and 24 did not wear any gloves at all. No one was positive for HBsAg or anti-HCV, though 73 (51.8%) and 17 (12.1%) workers were respectively positive for anti-HBs and anti-HBc. The positive rate of anti-HBc varied directly with worker age and experience. Of the 68 workers immunized with HBV vaccine, 51 (75%) were positive for anti-HBs. Of the 63 workers who were not so immunized, 17 (27%) were positive for anti-HBs and 15 of these were also positive for anti-HBc. Immunized workers were more protected against HBV infection than non-immunized workers, indicating that HBV vaccine was a useful measure for protection against the infection. The anti-HBc positive rate was significantly higher among dental care workers than general blood donors, suggesting that frequency of exposure to HBV was greater in dental care workers. HBV vaccination should be made compulsory for all dental care workers who handle sharp instruments.

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Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to HBsAg; anti-HBc, antibody to hepatitis B core antigen; HCV, hepatitis C virus; anti-HCV, anti-bodies to HCV; HCC, hepatocellular carcinoma; CLEIA, chemiluminescent enzyme immunoassay

Key words: dentists, hepatitis B virus, hepatitis C virus, vaccine

Introduction

It is important to protect dental care workers (who perform invasive procedures daily) from nosocomial, blood-transmissible infections of the hepatitis B virus (HBV) and hepatitis C virus (HCV). There are ~1.5 million persistent HBV carriers and 2 million persistent HCV carriers in Japan. These carriers may develop hepatocellular carcinoma (HCC) decades later. The incidence of HCC continues to increase in Japan and ~80 and 10% of HCC are due to HCV and HBV, respectively (1). Treatment methods for hepatitis C and hepatitis B are now well established, continue to improve annually and their effects are dramatic.

The worldwide HBV infection rate is higher in dentists than in the general population: 6 times higher in the USA, 4 times higher in Germany and 2.5 times higher in Japan. The incidence of HBV infection among dentists is 10.8% in Brazil (2), 9% in the USA (3) and 7% in Germany (4). Among medical care workers, dentists have the highest incidence of HBV infection and this incidence increases with the length of clinical experience of the dentist (5,6). An investigation conducted in 1978 in Japan found approximately half of dentists with 5 or more years of clinical experience were infected with HBV or had a history of HBV infection (7). A study of 998 dentists conducted in 17 regions throughout Japan from 1978 to 1982 reported that 37 (3.7%) were hepatitis B surface antigen (HBsAg)-positive and 420 (42.1%) were antibody to HBs (anti-HBs)-positive (8). The results indicated that infection occurred at work without the dentists' knowledge. Thus dental care workers should be advised to receive a hepatitis B vaccine and it should be confirmed if they have acquired immunity to HBV.

What is the HCV infection rate in dentists in Japan? The anti-HCV-positive rate was 2.6% (10/382) according to the seroepidemiological survey of Shinozaki *et al* who used frozen-preserved serum obtained from dentists between 1986 and 1994 (9). However, the status of HCV infection was unclear, as the mean age of subject dentists and other information were not recorded. In New York city, the positive rate of anti-HCV was clearly higher among oral surgeons (9.3%) and other dentists (1.75%) than blood donors (0.14%) (10). The finding shows that morbidity in dentists differs by specialties.

In recent years, the infection rate in dentists in Japan remains unclear. The last estimates were made in the 1980s and

1990s, before more sensitive tests became available. Viral levels have never been measured (7-9). The status of hepatitis viral infection in dental care workers in the northern part of Kyushu, where the infection rates are the highest in Japan should be determined in order to assess the extent to which further health measures are needed to protect and maintain the health of dental care workers.

The present study screened for the presence of HBV and HCV infections in dental care workers in the Fukuoka prefecture (northern Kyushu). Since viral hepatitis is treatable, this investigation could contribute to the health maintenance of dental care workers.

Patients and methods

Patients. Participants included 141 dentists belonging to the X Dental Association in the Fukuoka prefecture and dental care workers (dental hygienists, assistants, mechanics and clerks) employed at dental clinics. Each member was notified by mail about the study before the examination. The examination was performed on 2 days (September 22 and 27, 2007).

Methods. Each participant gave informed consent and had a blood sample taken. An oral surgery specialist interviewed the subjects. Items of inquiry included gender, age, occupation, years employed as a dental care worker, disposable glove use, history of jaundice, history of blood transfusion, clinical history of liver diseases, family history of liver disease and hepatitis B vaccination status.

Viral markers of hepatitis were measured by chemiluminescent enzyme immunoassay (CLEIA) including HBsAg, anti-HBs and anti-HBc and by solid phase RIA including anti-HCV. When the serum was HBsAg-positive, HBeAg (CLEIA), anti-HBe (CLEIA), HBV DNA level (PCR method) and HBV genotype (PCR method) were assayed; when the serum was anti-HBc-positive, the anti-HBc IgM and HBV DNA level were assayed; and when the serum was anti-HCV-positive, RT-PCR was carried out to determine quantitative HCV RNA and HCV genotype.

Results were mailed to each participant. Ethical guidelines for the research were observed closely in order to protect participant confidentiality.

Results

There were 141 (43 males and 98 females) participants. Table I shows 43 were in their 20s, 35 in their 30s, 36 in their 40s, 17 in their 50s, 7 in their 60s, 2 in their 70s and 1 in his 80s. There were 42 dentists, 35 dental hygienists, 41 dental assistants, 8 dental mechanics and 15 clerks. Six subjects had a clinical history of liver disease that was unrelated to HBV or HCV infection.

As for hepatitis B vaccination, 68 (48.2%) were and 63 (44.7%) were not vaccinated. Dentists were the largest vaccinated group (39.7%, 27/68) and dental assistants were the largest unvaccinated group (34.9%, 22/63).

Regarding disposable glove use, only 9 people reported use of new gloves with every new patient. The highest number of people (36/141) said that they changed gloves only when the old pair of gloves were torn. Twenty-four workers did not wear gloves.

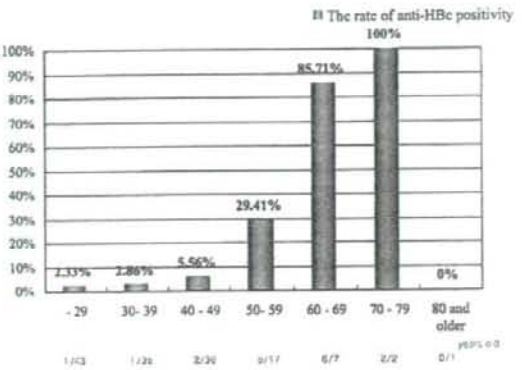

Figure 1. The rate of anti-HBc in 141 subjects classified according to age brackets. The rate of anti-HBc positivity increased with increased age.

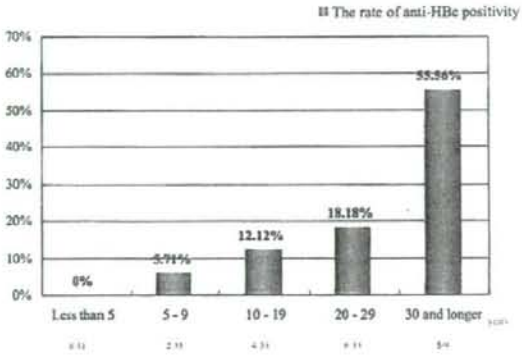

Figure 2. The rate of anti-HBc in 141 subjects classified according to years of experience in dental care. The rate of anti-HBc positivity increased with the number of years of dental care experience.

In hematological tests, no subjects were HBsAg-positive or anti-HCV-positive (Table II). However, 73 (51.8%) subjects were anti-HBs-positive and 17 (12.1%) were anti-HBc-positive. The rates of anti-HBc positivity increased with age: 85.7% of subjects in their 60s and 100% of subjects in their 70s (Fig. 1). The rate of anti-HBc positivity increased with the number of years of dental care experience (Fig. 2). As Table II shows, anti-HBs turned positive, indicating vaccine effectiveness, in 75% (51/68) of the vaccinated group and 27% (17/63) of the unvaccinated group. Fifteen of these 17 were anti-HBc-positive, indicating that these 15 were infected with HBV in the past.

Most (52.9%) of the 17 anti-HBc-positive subjects were dentists (Table III). The largest proportion of the anti-HBc-positive subjects were in their 60s (35.3%) and had 20 years of experience working in dentistry. Sixteen of the HBc-positive subjects (94.1%) were anti-HBs-positive. However, no HBV DNA was detected in the blood.

Table I. Background factors of 141 subjects classified by hepatitis B vaccination status.

	Total	Vaccination yes		Vaccination no		During vaccination (or Drop-out)		Unknown	
	(n)	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
		68	48.2	63	44.7	4	2.8	6	4.3
Sex									
Male	43	25	36.8	15	23.8	2	50.0	1	16.7
Female	98	43	63.2	48	76.2	2	50.0	5	83.3
Age									
29 years old	43	18	26.5	19	30.2	2	50.0	4	66.7
30-39	35	20	29.4	13	20.6	0	0.0	2	33.3
40-49	36	24	35.3	12	19.0	0	0.0	0	0.0
50-59	17	4	5.9	11	17.5	2	50.0	0	0.0
60-69	7	2	2.9	5	7.9	0	0.0	0	0.0
70-79	2	0	0.0	2	3.2	0	0.0	0	0.0
80 and older	1	0	0.0	1	1.6	0	0.0	0	0.0
Type of occupation									
Dentist	42	27	39.7	12	19.0	2	50.0	1	16.7
Dental hygienist	35	19	27.9	14	22.2	0	0.0	2	33.3
Dental assistant	41	15	22.1	22	34.9	2	50.0	2	33.3
Dental mechanic	8	4	5.9	4	6.3	0	0.0	0	0.0
Clerk	15	3	4.4	11	17.5	0	0.0	1	16.7
Years engaged in dental care									
<5 years	31	10	14.7	16	25.4	1	25.0	4	66.7
5-9	35	18	26.5	14	22.2	1	25.0	2	33.3
10-19	33	21	30.9	12	19.0	0	0.0	0	0.0
20-29	33	17	25.0	15	23.8	1	25.0	0	0.0
30 and longer	9	2	2.9	6	9.5	1	25.0	0	0.0
How to equip oneself with disposable gloves (Plural answers were given)									
Wear new pair with every new patient	9	6	8.8	3	4.8	0	0.0	0	0.0
Wear new pair with every 2 to 3 patients	31	19	27.9	9	14.3	1	25.0	2	33.3
Wear new pair when old one is torn	36	18	26.5	15	23.8	1	25.0	2	33.3
Wear new pair about twice a day	7	5	7.4	2	3.2	0	0.0	0	0.0

Table I. Continued.

	Total (n)	Vaccination yes (n)	(%)	Vaccination no (n)	(%)	During vaccination (or Drop-out) (n)	(%)	Unknown (n)	(%)
	68	48.2	63	44.7	4	2.8	6	4.3	
Wear when invasive treatment is performed	30	14	20.6	15	23.8	0	0.0	1	16.7
Wear when infected patients are treated	29	8	11.8	19	30.2	1	25.0	1	16.7
Wear when instrument is washed	2	0	0.0	2	3.2	0	0.0	0	0.0
Do not use	24	10	15	12	19	1	25.0	1	17
History of jaundice									
Yes	1	1	1.5	0	0.0	0	0.0	0	0.0
No	133	66	97.1	57	90.5	4	100.0	6	100.0
Unknown	7	1	1.5	6	9.5	0	0.0	0	0.0
History of blood transfusion									
Yes	5	1	1.5	4	6.3	0	0.0	0	0.0
No	133	66	97.1	57	90.5	4	100.0	6	100.0
Unknown	3	1	1.5	2	3.2	0	0.0	0	0.0
Clinical history of liver diseases									
Yes	6[a]	5	7.4	1	1.6	0	0.0	0	0.0
No	135	63	92.6	62	98.4	4	100.0	6	100.0
Unknown	0	0	0.0	0	0.0	0	0.0	0	0.0
Family history of liver diseases									
Yes	9	4	5.9	4	6.3	1	25.0	0	0.0
No	124	63	92.6	54	85.7	3	75.0	4	66.7
Unknown	8	1	1.5	5	7.9	0	0.0	2	33.3

[a]Fatty liver (n=3), acute hepatitis A (n=1), primary biliary cirrhosis (n=1) and hepatitis (unknown cause).

Table II. Hepatitis virus markers classified by hepatitis B vaccination status.

Hepatitis B virus markers	Total	Vaccination yes (n)	(%)	Vaccination no (n)	(%)	During vaccination (or Drop-out) (n)	(%)	Unknown (n)	(%)
		68	48.2	63	44.7	4	2.8	6	4.3
HBsAg									
Positive (+)	0	0	0.0	0	0.0	0	0.0	0	0.0
Negative (-)	141	68	0.0	63	0.0	4	0.0	6	0.0
Anti-HBs									
Positive (+)	73	51	75.0	17	27.0	2	50.0	3	50.0
Negative (-)	68	17	25.0	46	73.0	2	50.0	3	50.0
Anti-HBc									
Positive (+)	17	1	1.5	16	25.4	0	0.0	0	0.0
Negative (-)	124	67	98.5	47	74.6	4	100.0	6	100.0
Anti-HBs positive (+)									
Anti-HBc positive (+)	16	1	1.5	15	23.8	0	0.0	0	0.0
Anti-HBc negative (-)	57	50	73.5	2	3.2	2	50.0	3	50.0
Anti-HBs negative (-)									
Anti-HBc positive (+)	1	0	0.0	1	1.6	0	0.0	0	0.0
Anti-HBc negative (-)	67	17	25.0	45	71.4	2	50.0	3	50.0
Anti-HCV									
Positive (+)	0	0	0.0	0	0.0	0	0.0	0	0.0
Negative (-)	141	68	0.0	63	0.0	4	0.0	6	0.0

Table III. Breakdown of results classified by anti-HBc (+) and anti-HBc (-).

	Subjects with anti-HBc (+) 17		Subjects with anti-HBc (-) 124	
	(n)	(%)	(n)	(%)
Hepatitis B vaccine				
Vaccination yes	1	5.9	67	54.0
Vaccination no	16	94.1	47	37.9
During vaccination (or Drop-out)	0	0.0	4	3.2
Unknown	0	0.0	6	4.8
Sex				
Male	9	52.9	34	27.4
Female	8	47.1	90	72.6
Age				
29 years old	1	5.9	42	33.9
30-39	1	5.9	34	27.4
40-49	2	11.8	34	27.4
50-59	5	29.4	12	9.7
60-69	6	35.3	1	0.8
70-79	2	11.8	0	0.0
80 and older	0	0.0	1	0.8
Type of occupation				
Dentist	9	52.9	33	26.6
Dental hygienist	2	11.8	33	26.6
Dental assistant	2	11.8	39	31.5
Dental mechanic	0	0.0	8	6.5
Clerk	4	23.5	11	8.9
Years engaged in dental care				
<5 years	0	0.0	31	25.0
5-9	2	11.8	33	26.6
10-19	4	23.5	29	23.4
20-29	6	35.3	27	21.8
30 and longer	5	29.4	4	3.2
How to equip oneself with disposable gloves (Plural answers were given)				
Wear new pair with every new patient	1	5.9	8	6.5
Wear new pair with each 2 to 3 patients	1	5.9	30	24.2
Wear new pair when old one is torn	0	0.0	36	29.0
Wear new pair about twice a day	0	0.0	7	5.6
Wear when invasive treatment is performed	7	41.2	23	18.5
Wear when infected patients are treated	9	52.9	20	16.1
Wear when instrument is washed	0	0.0	2	1.6
Do not use	4	23.5	20	16.1
History of jaundice				
Yes	0	0.0	1	0.8
No	17	100.0	116	93.5
Unknown	0	0.0	7	5.6
History of blood transfusion				
Yes	1	5.9	4	3.2
No	15	88.2	118	95.2
Unknown	1	5.9	92	1.6

Table III. Continued.

	Subjects with anti-HBc (+) 17		Subjects with anti-HBc (-) 124	
	(n)	(%)	(n)	(%)
Clinical history of liver diseases				
Yes	1	5.9	5	4.0
No	16	94.1	119	96.0
Unknown	0	0.0	0	0.0
Family history of liver diseases				
Yes	2	11.8	7	5.6
No	15	88.2	109	87.9
Unknown	0	0.0	8	6.5
HBsAg				
Positive (+)	0	0.0	0	0.0
Negative (-)	17	100.0	124	100.0
Anti-HBs				
Positive (+)	16	94.1	57	46.0
Negative (-)	1	5.9	67	54.0
Anti-IgM-HBc				
Positive (+)	0	0.0	-	-
Negative (-)	17	100.0	-	-
HBV DNA quantitative measurement				
≥2.6 log/ml	0	0.0	-	-
<2.6 log/ml	17	100.0	-	-

Discussion

HBV infection is transmitted mostly through blood and body fluid as a result of bites, administration of blood preparations, sexual activities and mother-infant transmission. The principal route of HCV infection is through blood. Medical care workers are always at risk of infection as they are exposed to contaminated fluids from needle sticks and infected blood droplets. Hepatitis B immune globulin (HBIG) has been used since 1981 and hepatitis B vaccination since 1985 (whole virus) and 1988 (recombinant) to prevent infection.

Dental care workers are often exposed to blood because of stomatorrhagia and the use of sharp instruments (11). Meticulous measures should be taken to protect against the spraying of saliva, which contains blood inside the examination room (12,13). Our previous study reported that saliva from HCV carriers contained HCV RNA before and after scaling of dental calculus (14). HCV RNA was detected in exudates from gingival crevicular fluid and on materials used for making dental impressions, a work bench, an air turbine dental handpiece, holders, suction units, forceps, dental mirrors and cutting bar (15-17). HCV RNA was still detectable on the surface of dental instruments several days after the HCV carriers received treatment (18). Although their risk of infection is high, dental care workers are obligated to prevent cross infection (i.e., from dental care workers to patients and patients to patients). Although there are no documented cases of HCV transmission from dentists to patients, there is one case of the transmission of HBV by an oral surgeon (19).

Whether the disease is contracted depends on the levels of the virus in the blood, source of contamination, route of contact and blood volume transfused (20). The rate of acquiring HBV infection through HBV-contaminated needles is high [12% (21) to 60% (22) in unvaccinated persons]. Wounds caused by needles that are contaminated with HBsAg- and HBeAg-positive blood are associated with a 22-31% risk of developing hepatitis B and a 37-62% probability of establishing HBV infection (23). Wounds caused by needles contaminated with HBsAg-positive and HBeAg-negative blood are associated with a 1-6% risk of developing hepatitis B and a 23-37% probability of establishing HBV infection (21). However, infection can be prevented by HB vaccination and the administration of HBIG after these accidents occur.

Accidental prick with a needle contaminated with HCV-positive blood caused HCV infection in ~1.4 (24) to 10% (25) of cases. The probability of infection due to contaminated needle sticks is lower for HCV than HBV. However, the high risk of developing HCC through horizontal infection of HCV is a concern to often-exposed dentists. Feldman and Schiff found that hepatitis morbidity was 6.7% in dentists and 21% in oral surgeons in the State of Florida, USA (26). Although the risk of hepatitis among dentists is high, a long-term cohort study by Tanaka *et al* reported that liver cancer risk was no higher in Japanese dentists than in the general population (27).

In the present investigation, 51 of the 68 recipients of the HBV vaccine were anti-HBs-positive, indicating that 75% of vaccinated subjects developed an antibody to HBV infection. Of the 63 unvaccinated subjects, 16 (25.4%) were anti-HBc-positive and had no clinical history of HBV-related liver diseases, suggesting that they had been transiently and inapparently infected with HBV in the past. Only 1 (1.5%) of the 68 vaccinated subjects was anti-HBc-positive, indicating the protection rate against HBV infection was higher in vaccinated than unvaccinated subjects and that vaccination was a useful protective measure.

The Japanese Red Cross introduced the Hemagglutination Inhibition Test (HI) in 1989 for the screening of anti-HBc (28) and the Nucleic Acid Amplification Test (NAT) in 1999 for the screening of HBV, HCV and HIV in blood that was HBsAg-, anti-HBc-, anti-HCV- and anti-HIV-negative with ALT values <61 IU/l, dramatically increasing the safety of blood transfusion (29).

In Fukuoka and Kitakyushu Red Cross, 3,647 (1.1%) of 323,799 blood donors screened between April 2003 and October 2004 were anti-HBc-positive. Of these 3,647, a total of 445 were HBsAg-positive (30). In the remaining 3,202 anti-HBc-positive, HBsAg-negative donors, the rates of seroconversion to anti-HBc increased with age (0.10, 0.23, 0.57, 1.38, 2.10 and 2.29%, respectively, in age groups 16-19, 20-29, 30-39, 40-49, 50-59 and 60-69).

Seroconversion to anti-HBc occurred at a significantly higher rate in dental care workers (12.1%) than blood donors ($p<0.05$).

Anti-HBc is a marker of latent hepatitis B (31,32). In previous years, it has been reported that HBV infection was transmitted through a liver transplanted from an anti-HBc-positive donor (32). HBV DNA has been detected in the serum of patients recovered from acute hepatitis B (33). Infection of latent HBV has been associated with the onset of HCV-related HCC (34,35). Therefore, from the standpoint of health safety, the prevalence of latent HBV infection among dental care workers must be acknowledged.

Of the 63 unvaccinated subjects, only 4.8% changed gloves to a new pair for each new patient and 19% never wore gloves. Since dental care workers have a high risk of exposure to the hepatitis virus, a compulsory vaccination for the hepatitis B virus is desirable for all dental care workers. In Japan, hepatitis B vaccination is voluntary. However, from the standpoint of effectiveness and safety and to reduce infection risk, it is important to vaccinate these workers.

Regrettably, no hepatitis C vaccine or immunoglobulin has been developed to prevent HCV infection. Although no persistent carriers of HBV and HCV were detected in the present investigation, the rate of infection is higher in the western portion of Japan, especially in the Saga and Fukuoka prefectures, than eastern Japan. Therefore, further precautions must be taken.

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